Partition of Environmental Chemicals between Maternal and Fetal Blood and Tissues

Supporting Information, 13 pages, including 3 tables:
Sampling and chemical analysis methods, and tables with results on analytes that did not comply with the overall average partition ratio

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S1. Sampling and chemical analyses

Sampling. Maternal blood—from a cubital vein of the mother during week 32 of the pregnancy (last antenatal examination, non-fasting)—was collected in an evacuated Vacutainer tube (10 mL) without anticoagulant (Becton-Dickinson, Rutherford, New Jersey, USA). Serum was harvested after centrifugation of the coagulated sample. Cord blood was collected immediately after the cord had been clamped by using 10-mL Abbott syringes with Teflon-lined pistons (Abbott Ireland Ltd, Sligo, Ireland). Both whole blood and serum were obtained. All maternal and cord serum samples were transferred into Minisorb tubes (Nunc, Roskilde, Denmark) for storage.

After cord blood collection for whole blood and serum specimens, a minimum of 10 cm of cord tissue was taken close to the umbilicus of the infant and placed into a Minisorb tube. Subsequently, the placenta was secured, and multiple cube-like pieces were cut from both peripheral and central lobes. Visible calcium deposits and white infarct areas were avoided. The first approximately 4 mm from the maternal side—i.e., the basal decidua—was removed from each cube. Any marginal tissue, which is entirely of maternal origin, was removed as well, as was the chorionic plate; hence, the specimen collected consisted of placental/trophoblastic tissue of fetal origin. Maternal blood is easier to remove than is fetal blood, and repeated washings were conducted by use of saline. Each specimen was transferred to a Minisorb tube.

During days 3–5 after delivery, while the mother was still at the hospital, transitional milk was collected directly into acid-rinsed 250 ml polyethylene bottles (Kartell, Einasco, Milano, Italy). Collection of a minimum of 50 mL from each mother was attempted. All liquid and tissue samples were immediately frozen at -20°.

For mercury measurements, hair samples of at least 100 mg (usually of 5–8 cm length) were cut with scissors close to the root from the occipital area; the hair sample was tied with a cotton thread and saved in a small, marked envelope. For analyses of organochlorine compounds, 2 g of serum and milk were used. To compensate for lower lipid contents, about 5 g of placenta and cord tissue were applied for these analyses.

PCBs, chlorinated pesticides, PBDEs, and PBBs. The lipophilic organohalogen compounds measured and their abbreviations are listed in Tables S1-S3. The pretreatment of samples before analysis at CDC varied with the matrix. The methodology used for processing serum samples (*1, 2*) included automatic fortification with internal standards as well as addition of formic acid and water for denaturation and dilution of the samples by use of a Gilson 215 liquid handler (Gilson Inc.; Middleton, WI). The samples were thereafter extracted by solid phase extraction (SPE) through use of a Rapid Trace (Caliper Life Sciences; Hopkinton, MA) modular SPE system. Removal of co-extracted lipids was performed on a silica: silica/sulfuric acid column through use of the Rapid Trace equipment for automation. Final analytical measurement of the target analytes was performed by gas chromatography isotope dilution high resolution mass spectrometry (GC-IDHRMS) employing a MAT95XP (ThermoFinnigan MAT, Bremen, Germany) instrument.

The method used for processing milk samples (*1*) included solid phase dispersion on Hydromatrix (Varian Inc.; Walnut Creek, CA) and automatic fortification with internal standards by use of a Gilson 215 liquid handler (Gilson Inc.; Middleton, WI). The samples were extracted by use of the automated SPE system Rapid Trace (Zymark; Hopkinton, MA). Removal of co-extracted lipids was performed on a silica:silica/ sulfuric acid column using the Rapid Trace equipment for automation. Final analytical measurement of the target analytes was performed by gas chromatography isotope dilution high resolution mass

spectrometry (GC- IDHRMS) employing a MAT95XP (ThermoFinnigan MAT, Bremen, Germany) instrument.

The method used for tissue samples (cord and placenta) is outlined in Jensen et al. (*3*), and that method was used with modification. Tissue samples (5 g) were fortified with internal standards and homogenized in (hexane: acetone 7:2; 20 mL). The samples were centrifuged and the organic solvent transferred to another vial. The sample was re-suspended in hexane: methyl *tert*-butyl ether (MTBE) (9:1; 10 mL) and centrifuged, and the organic solvents were combined. The samples were extracted once more with hexane: MTBE. The combined organic phases were partitioned against 0.1M H_3PO_4 in 1% NaCl and thereafter evaporated to dryness (for lipid weight determination). Removal of co-extracted lipids was performed on a silica:silica/sulfuric acid column using the Rapid Trace equipment for automation.

Among the polychlorinated biphenyls, the following congeners were determined: 18, 28, 44, 49, 52, 66, 74, 87, 99, 101, 105, 110, 118, 128, 138/158, 146, 149, 151, 153, 156, 157, 167, 170, 172, 177, 178, 180, 183, 187, 189, 194, 195, 196/203, 199, 206, and 209. The persistent pesticides included pentachlorobenzene (PCBz); hexachlorobenzene (HCB); β-hexachlorocyclohexane (β-HCH); γ-hexachlorocyclohexane (lindane, γ-HCH); octachlorostyrene; oxychlordane; trans-nonachlor; 2,2-bis(4-chlorophenyl)-1,1-dichloroethene (*p,p'*-DDE); 2-(4-chlorophenyl)-2-(2-chlorophenyl)- 1,1,1-trichloroethane (*o,p'*-DDT); 2,2-bis(4-chlorophenyl)-1,1,1-trichloroethane (*p,p'*-DDT) mirex; and toxaphene congeners: 2-endo,3-exo,5-endo,6-exo,8,8,10,10-octachlorobornane (P26), 2-endo,3-exo,5-endo,6-exo,8,8,9,10,10-nonachlorobornane (P50), 2,2,5,5,8,9,9,10,10-nonachlorobornane (P62). The following brominated flame retardants (BFRs) were measured: 2,4,4'-tribromodiphenyl ether (BD-28), 2,2',4,4'-tetrabromodiphenyl ether

(BDE-47), 2,2',3,4,4'-pentabromodiphenyl ether (BDE-85), 2,2',4,4',5-pentabromodiphenyl ether (BDE-99), 2,2',4,4',6-pentabromodiphenyl ether (BDE-100), 2,2',4,4',5,5'-hexabromodiphenyl ether (BDE-153), 2,2',4,4',5,6'-hexabromodiphenyl ether (BDE-154), 2,2',3,4,4',5',6-heptabromodiphenyl ether (BDE-183), and 2,2',4,4',5,5'-hexabromobiphenyl (BB-153).

Analytical measurements of the 58 target analytes were performed by gas chromatography isotope dilution high resolution mass spectrometry (GC- IDHRMS) employing a MAT95XP (ThermoFinnigan MAT, Bremen, Germany) instrument (*3*). All organohalogen laboratory results were reviewed and approved by a quality assurance officer to assure that they conform to acceptable quality standards. However, the tissue samples analyzed were not analyzed with quality assurance/quality control (QA/QC) samples because there are no QC samples available for cord and placenta. The tissues were, however, measured by isotope-dilution high resolution mass spectrometry, which is the most accurate and precise method of quantification for such chemicals. The isotope-dilution method includes the spiking of the tissue with isotopically labeled internal standards that are chemically identical to the analytes being measured. Since the matrix analytes and isotopically labeled analytes are chemically identical, the ratio of the analytes is identical throughout the extraction and cleanup steps. This ratio is used to calculate the quantitative amount of analytes present in the tissues.

PCDDs, PCDFs, coplanar PCBs in maternal serum and milk. Samples were analyzed at CDC and spiked with $^{13}C_{12}$-labeled internal standards followed by C_{18} solid-phase extraction (SPE) (*4*) and a multicolumn automated cleanup and enrichment procedure using a Fluid Management Systems Power-Prep/6. An analytical run was comprised of two method blanks, eight unknown samples, and two quality control samples. PCBs were

eluted from the AX-21 carbon in the forward direction with hexane and dichloromethane (1:1), and PCDDs/PCDFs/cPCBs were eluted in the reverse direction with toluene. One µL of dodecane "keeper" was added to each of the eluants and the solvent was evaporated to about 350 µL by use of a Caliper TurboVap II. Residual solvent was transferred to silanized autosampler vials and evaporated to one µL. Before analysis by high-resolution gas chromatography (HRGC) and high-resolution mass spectrometry (HRMS), the vials were reconstituted with 5-µL of 13C- labeled external standard in nonane. A Leap Technology GC Pal autosampler was used to make 2-µL injections into an Agilent 6890 gas chromatograph (GC). The GC was operated in the splitless injection mode with a flow of 1 mL/min He through a DB-5ms column (30 m x 0.25 mm x 0.25 µm film). Selected congeners were quantified by isotope-dilution mass spectrometry (IDMS) using selected ion monitoring (SIM) at 10,000 resolving power (10% valley) on two Thermo Electron MAT 95 XP (5kV) magnetic sector field mass spectrometers (with "sensitivity" upgrade part# 1150760 installed) operated in the electron impact (EI) mode at 40 eV. The HRMS quantification scheme employed for seventeen 2,3,7,8-substituted PCDDs/PCDFs and three coplanar PCBs used six MID descriptor groups.

Sums of congener groups were calculated under the assumption that non-detectable results were zero. Dioxin equivalent concentrations were calculated by use of WHO equivalency factors (2005)

Lipid determination. For standardization of analytical results for lipophilic substances, their concentrations were expressed in regard to the lipid content. Total lipid contents were determined gravimetrically for milk and for placenta and cord tissue. For maternal and cord serum, the lipid concentration was calculated from the concentrations of total cholesterol and triglycerides, as previously described (*5*). Although this formula has

not been tested for cord serum, it relies on actual measurements of total cholesterol and triglycerides as major lipid components in serum.

Perfluorinated compounds. Analyses of serum samples for PFCs (perfluorohexane sulfonic acid (PFHxS), perfluorooctanic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA)) were performed at the University of Southern Denmark, Denmark, by the method described by Kuklenyik et al. (*6*), with minor modification. The LC-MS/MS system consisted of a TSQ Quantum Ultra with heated-electrospray ionisatizion (H-ESI) operated in negative-ion mode and connected to an Accela HPLC pump and a CTC autosampler (Thermo Fisher Scientific, Palo Alto, CA). The separation was performed on a Betasil C8 50 x 3 mm (5 µm) column from Thermo Fischer Scientific, Palo Alto, CA) by use of a gradient system (mobile phase A: 20 mM ammonium acetate in water, pH = 4; mobile phase B: methanol). The mass transitions, collision energies, and tube lens settings were optimized individually for all the compounds. The compounds were quantitated by use of the 13C-isotope-labeled analogues as internal standards (except for PFHxS, which was calculated by use of the internal standard for PFOA). The extraction of the serum samples was performed by manual solid-phase extraction, and an aliquot of 30 µL was injected into the LC-MS/MS system. The limit of detection was < 0.05 ng/mL for PFHxS, PFOA, PFNA, and PFDeA, and it was < 0.1 ng/mL for PFOS.

The analyses of the milk specimens were conducted at CDC, through use of a modification of the method used for measuring PFCS in serum (*7*). Briefly, 275 µL of 0.1 M formic acid and 25 µL of internal standard solution were added to 100 µL of milk. The spiked milk was vortex-mixed and sonicated, then injected into a Symbiosis on-line SPE-HPLC system (Spark Holland, Plainsboro, NJ), allowing for the preconcentration of the

target PFCs on a Polaris C18 cartridge (7 mm, 10 × 1 mm; Spark Holland). This column was automatically positioned in front of a Betasil C8 HPLC column (3 x 50 mm, 5 µm; ThermoHypersil-Keystone, Bellefonte, PA) for the chromatographic separation of the PFCs (20mM ammonium acetate (pH 4) in water (mobile phase A) and methanol (mobile phase B)). Detection and quantification used negative-ion TurboIonspray ionization-tandem mass spectrometry on an API 4000 mass spectrometer (Applied Biosystems, Foster City, CA). The limits of detection ranged between 0.1 and 0.2 ng/mL, depending on the analyte. In addition to the calibration standards, blanks and quality control (QC) materials were analyzed along with the samples to ensure the accuracy and reliability of the data across time. Results for quality control materials were evaluated by use of standard statistical probability rules (*8*).

Trace elements. All samples were measured at the University of Southern Denmark by atomic absorption; tissue samples were freeze-dried before analysis. For lead, cadmium, and selenium, we used a Perkin-Elmer model 5100 atomic absorption spectrometer with Zeeman background correction, HGA-600 graphite furnace, and an AS-60 autosampler (Perkin-Elmer, Norwalk, CT, USA). All samples were analyzed in duplicate. Average detection limits were 0.03 µg/L for cadmium and 1.7 µg/L for lead; for approximately 0.1 g of dried tissue dissolved in 500 µL of dilute acid, these limits correspond to about 0.15 ng/g and 8.5 ng/g for tissue concentrations of cadmium and lead, respectively. Mercury was determined by duplicate analyses using flow-injection cold-vapor atomic absorption spectrometry after digestion of the sample in a microwave oven (*9*).

Table S1. Average partition ratios for brominated substances that deviated from the overall mean partition ratios (medians in parenthesis if the correlation coefficient between paired samples was below 0.7).

Analyte and abbreviation*	Cord serum	Cord tissue	Placenta	Milk
BDE47 (2,2',4,4'-tetrabromodiphenyl ether)	-	1.10	(0.48)	2.5
BDE99 (2,2',4,4',5-pentabromodiphenyl ether)	-	-	(0.33)	+
BDE100 (2,2',4,4',6-pentabromodiphenyl ether)	-	-	(0.32)	2.7
BB153 (2,2',4,4',5,5'-hexabromobiphenyl)	-	-	+	0.89
BDE153 (2,2',4,4',5,5'-hexabromodiphenyl ether)	0.34	(0.61)	+	+

+ included in overall ratio; - insufficient number of observations

* BDE85 and BDE154 were disregarded due to fewer than five pairs of detectable levels

Table S2. Average partition ratios for chlorinated pesticides that deviated from the overall mean partition ratios (medians in parenthesis if the correlation coefficient between paired samples was below 0.7).

Analyte (Abbreviation)*	Cord serum	Cord tissue	Placenta	Milk
Pentachlorobenzene (PCBz)	(10.8)	(1.11)	-	-
HCB (Hexachorobenzene)	+	0.72	0.56	+
β-HCH (β-hexachlorocyclohexane)	+	0.93	0.61	2.2
γ-HCH (γ-hexachlorocyclohexane)	(7.0)	(4.8)	(1.76)	(1.20)
OCS (Octachlorostyrene)	-	-	+	(1.12)
OXYCHLOR (Oxychlordane)	+	+	+	2.2
t-NONA (trans-nonachlor)	+	+	+	2.1
P50 (3-exo,5-endo,6-exo,8,8,9,10,10-nonachlorobornane)	+	+	(0.19)	2.5
P26 (2-endo,3-exo,5-endo,6-exo,8,8,10,10-octachlorobornane)	-	-	-	2.9
DDE (2,2-bis(4-chlorophenyl)-1,1-dichloroethene)	+	+	+	+
o,p-DDT (2-(4-chlorophenyl)-2-(2-chlorophenyl)- 1,1,1-trichloroethane)	+	-	(0.08)	+
p,p-DDT (2,2-bis(4-chlorophenyl-1,1,1-trichloroethane)	+	+	(0.08)	+
Mirex	+	+	+	0.88

+ included in overall ratio; - insufficient number of observations

* Toxaphene P62 was disregarded due to fewer than five pairs of detectable levels

Table S3. Average partition ratios for polychlorinated biphenyl (PCB) congeners that deviated from the overall mean partition ratios (medians in parenthesis if the correlation coefficient between paired samples was below 0.7). Congener numbering is according to Ballschmiter (*10*).

Analyte*	Cord serum	Cord tissue	Placenta	Milk
PCB28	2.2	(2.3)	1.80	+
PCB44	(34)	(35)	(63)	-
PCB49	2.9	(16)	(21)	-
PCB52	-	(25)	(44)	2.7
PCB66	1.00	(1.8)	(3.1)	2.1
PCB74	+	0.93	(0.67)	+
PCB99	+	0.92	(0.65)	+
PCB101	+	(9.9)	(19)	+
PCB105	+	(0.64)	(0.98)	+
PCB118	+	0.97	(0.98)	+
PCB128	+	(0.67)	(0.87)	+
PCB138/158	+	+	0.64	+
PCB146	+	+	0.51	+
PCB149	-	(3.3)	(10)	+
PCB151	+	1.28	(1.42)	+
PCB153	+	+	0.51	+

PCB156	+	+	0.56	+
PCB157	+	+	0.51	+
PCB172	1.22	0.86	0.77	2.6
PCB189	-	+	+	+
PCB194	+	0.24	+	0.85
PCB196/203	+	+	+	0.87
PCB201	+	+	+	0.89
PCB206	+	0.20	+	0.56
PCB209	0.33	0.20	+	0.27

+ included in overall ratio; - insufficient number of observations

* PCB congeners 18, 87, 110 were disregarded due to fewer than five pairs of detectable levels. PCB congeners 167, 170, 177, 178, 180, 183, 187, and 195 contributed to the overall mean for all sample pairs and are therefore left out as well.

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